

This Page Is Inserted by IFW Operations
and is not a part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

**As rescanning documents *will not* correct images,
please do not report the images to the
Image Problem Mailbox.**

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C07H 19/06, C07D 239/54, A61K 31/70, 31/505	A1	(11) International Publication Number: WO 95/12606 (43) International Publication Date: 11 May 1995 (11.05.95)
(21) International Application Number: PCT/GB94/02428 (22) International Filing Date: 4 November 1994 (04.11.94) (30) Priority Data: 9322795.7 5 November 1993 (05.11.93) GB (71) Applicant (for all designated States except US): THE WELL-COME FOUNDATION LIMITED [GB/GB]; Unicom House, 160 Euston Road, London NW1 2BP (GB). (72) Inventors; and (75) Inventors/Applicants (for US only): BOYD, Frank, Leslie [US/US]; 8609 Timberwind Drive, Raleigh, NC 27615 (US). KRENITSKY, Thomas, Anthony [US/US]; 106 Laurel Hill Road, Chapel Hill, NC 27514 (US). (74) Agent: ROLLINS, Anthony, John; The Wellcome Foundation Limited, Langley Court, Beckenham, Kent BR3 3BS (GB).		(81) Designated States: AU, BR, CA, CN, CZ, FI, GE, HU, JP, KR, KZ, LT, NO, NZ, PL, RU, SI, SK, UA, US, UZ, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report.</i>
(54) Title: 5-FLUOROURACIL DERIVATIVES (57) Abstract Novel compounds of formula (I): X-L-Y, (II): X-(L-Y) _n and (III): X-L-(Y) _n wherein X is a uracil reductase inactivator or prodrug thereof, Y is 5-fluorouracil or a prodrug thereof, L is a linking group covalently linked to both X and Y and n is at least 2, the use of such compounds in medicine, in particular cancer chemotherapy, pharmaceutical compositions comprising such compounds, and a process for the preparation of such compounds are disclosed.		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LU	Luxembourg	TD	Chad
CS	Czechoslovakia	LV	Latvia	TG	Togo
CZ	Czech Republic	MC	Monaco	TJ	Tajikistan
DE	Germany	MD	Republic of Moldova	TT	Trinidad and Tobago
DK	Denmark	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				

5-FLUOROURACIL DERIVATIVES

The present invention relates to compounds which comprise certain enzyme inactivators which are useful in medicine, particularly cancer chemotherapy, covalently linked to antineoplastic agents, particularly 5-fluorouracil (5-FU).

5-Fluorouracil has been used in cancer chemotherapy since 1957. Sensitive tumours include breast cancer, gastrointestinal malignancies, and cancers of the head and neck; 5-fluorouracil is also used as a radiation sensitiser. 5-Fluorouracil is metabolised rapidly in the liver (half-life between 8 and 20 minutes) by the enzyme dihydrothymidine dehydrogenase (uracil reductase). It has been reported (Cancer Research 46, 1094, 1986) that 5-(2-bromovinyl)-uracil (BVU) is an inhibitor of dihydrothymidine dehydrogenase which both retards the metabolism of 5-fluorouracil and enhances its antitumour activity. It has been reported that 5-(2-bromovinyl)-2'-deoxyuridine (which is metabolised *in vivo* to BVU) enhances the antitumour activity of 5-fluorouracil and 5-deoxy-5-fluorouridine, a prodrug of 5-fluorouracil (Biochemical Pharmacology 38; 2885, 1989).

WO92/04901 discloses 5-substituted uracil derivatives which are useful as inactivators of uracil reductase; they increase the level and half-life of 5-fluorouracil in plasma and enhance the activity of 5-fluorouracil. These derivatives also reduce the normally encountered variations of 5-fluorouracil plasma levels between subjects. 5-ethynyluracil is 100-fold more potent than BVU as an inactivator of uracil reductase.

It has now been found that useful compounds can be produced by covalently linking a uracil reductase inactivator moiety with a 5-fluorouracil moiety or a prodrug thereof.

Therefore a first aspect the present invention provides a compound of the formula:-



wherein X represents a uracil reductase inactivator or prodrug thereof, Y represents 5-fluorouracil or a prodrug thereof, and L is a linking group covalently linked to both X and Y. Preferably X is a 5-substituted or 5,6-dihydro-5-substituted uracil derivative,

- 2 -

the 5-substituent being bromo, iodo, cyano, halo-substituted C₁₋₄ alkyl, C₂₋₆ alkenyl, halo substituted C₂₋₆ alkenyl group, a C₂₋₆ alkynyl group, halo substituted C₂₋₆ alkynyl group.

These compounds when administered to a subject, for example a mammal such as a human, provide both a uracil reductase inactivator as well as the 5-fluorouracil antineoplastic agent itself. Thus, only a single active agent need be administered to a subject.

In addition, these compounds have the advantage that they can be administered orally. 5-Fluorouracil cannot normally be administered orally, as it is destroyed by uracil reductase in the gastro-intestinal tract. A single agent, which provides 5-FU and which can be administered orally, is now provided.

By a C₂₋₆ alkenyl or C₂₋₆ alkynyl group is meant a straight or branched chain alkenyl or alkynyl group, the latter including an alkenyl or alkynyl group substituted by a C₂₋₆ cycloalkyl group.

The halogen substituent on the alkenyl or alkynyl group is preferably bromo, chloro or iodo. Halo-substituted ethenyl and ethynyl groups are particularly preferred. Usually only one halo substituent will be present. Preferred halo-substituted alkenyl groups are substituted in the 1-position.

The uracil derivative moiety is preferably one wherein the 5-substituent is a C₂₋₆ alkynyl group (optionally halo-substituted), conveniently a C₂₋₄ alkynyl group and preferably an ethynyl or propynyl group. In preferred 1-halo-alkenyl and alkynyl derivatives the multiple bond is in the 1-position. Particularly preferred uracil derivatives which form part of the compounds as hereinbefore defined are 5-ethynyluracil and 5-propynyluracil. Other such inactivators include:-

- 5-cyanouracil
- 5-bromoethynyluracil
- 5-(1-chlorovinyl) uracil
- 5-iodouracil
- 5-hex-1-ynyluracil

- 3 -

5-vinyluracil
5-trifluoromethyluracil
5-bromouracil

Prodrugs of 5-fluorouracil are compounds which are metabolised in vivo to 5-fluorouracil and include 5-fluorouridine, 5-fluoro-2-deoxyuridine, 5-fluoro-2-deoxycytidine, 5'-deoxy-5-fluorouridine, 1-(2-tetrahydrofuryl)-5-fluorouracil and 1-C₁₋₈ alkylcarbamoyl-5-fluorouracil derivatives.

Prodrugs of the uracil reductase inactivators are compounds which are metabolised in vivo to the corresponding uracil reductase inactivator.

Such prodrugs include nucleoside analogues which contain a nucleobase corresponding to the above 5-substituted uracil compounds. For example nucleoside derivatives containing a ribose, 2'-deoxy-ribose, arabinose or other cleavable sugar portion, which may contain a 2' or 3'-substituent such as halo, e.g. chloro or fluoro; alkoxy; amino or thio. Specific examples of such nucleoside derivatives are 1-(6-O-arabinofuranosyl)-5-ethynyluracil and 1-(6-O-arabinofuranosyl)-5-prop-1-ynyluracil. Other uracil derivative prodrugs include 5-ethynyl-2-pyrimidinone.

The nature of the linking group, L, should be such that the active components are released once the compound has been administered to the subject.

Thus, breakdown of a compound of the invention will result in release of both 5-fluorouracil and the uracil reductase inactivator, or prodrugs thereof.

In a preferred embodiment both the 5-fluorouracil moiety and the uracil reductase inactivator moiety are presented in the form of nucleosides, these then being linked such that upon administration to the subject the linkage breaks down releasing the active components.

- 4 -

Suitable sugar moieties which together with 5-fluorouracil and the uracil reductase inactivator can form such nucleosides include ribose, 2'-deoxyribose, arabinose and 5'-Cl-2'-deoxyribose.

Examples of suitable linking groups, L, include succinate groups, pyrophosphate groups, tartrate groups, mono-, di- or tri- phosphate groups and amide groups, with succinate and phosphate groups in general being particularly suitable.

Examples of preferred compounds of the invention include:

5-ethynyl-5"-fluoro-3',3'''-O-succinylbis-(1-(2-deoxy- β -D-erythropentofuranosyl)uracil);

5-ethynyluridine-5-fluoro-1-(β -D-arabinofuranosyl)uracil succinic acid 5', 2'-diester;

5-fluoro-5''-(1-propynyl)-2', 2'''-O-succinylbis(1- β -D-arabinofuranosyl uracil); and

5'-chloro-2'-5'-dideoxy-5-fluorouridine 2'''-deoxy-5''-ethynyluridine (3'-5''')diphosphate.

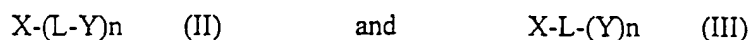
In general a suitable dose of a compound as hereinbefore described will be such as to provide a dose of 5-fluorouracil or a prodrug thereof in the range of 0.1 to 1000mg per kilogram body weight of the recipient per day, preferably in the range of 0.1 to 200mg per kilogram body weight per day. Most preferable is a dose in the range of 0.1 to 50mg per kilogram body weight per day. For the uracil derivative component a suitable dose is in the range of 0.01 to 50mg per kilogram body weight of the recipient per day, particularly 0.01 to 10mg/kg. More preferably the dose should be such as to provide 0.01 to 0.4mg per kilogram body weight per day as uracil derivative. Accordingly, a suitable dose of a compound as hereinbefore described will be in the range of 0.04 to 4000mg per kilogram body weight per day, preferably in the range of 0.04 to 800mg per kilogram body weight per day and most preferably in the range of 0.4 to 200mg per kilogram body weight per day.

Given the nature of the compound it will be seen that it is possible, by including further linking groups, to increase the number of 5-fluorouracil moieties, or prodrugs

- 5 -

thereof such that upon breakdown of the compound the dosages of 5-fluorouracil, or prodrug thereof, and uracil derivative fall within the abovementioned ranges.

Therefore the invention also provides compounds of the formulae (II) and (III):



Wherein X, L, and Y are as hereinbefore defined and n is at least 2. Preferably n is in the range 2-10.

For brevity, the term "a compound as hereinbefore defined" is used hereinafter to describe compounds of formula (I), (II) and (III).

In a further aspect the present invention provides a compound as hereinbefore defined for use in medicine, in particular for use in cancer chemotherapy.

In a still further aspect the present invention provides the use of a compound as hereinbefore defined in the manufacture of a medicament for use in cancer chemotherapy. The medicament may also be useful for the treatment of psoriasis, rheumatoid arthritis, or human papilloma virus infections.

In a further aspect the present invention provides a method for the treatment of cancer in a mammal comprising administering an effective amount of a compound as hereinbefore defined to said mammal. Alternatively, there is provided a method of reducing or inhibiting the tumour burden comprising administering to a mammal in need thereof an effective amount of a compound as hereinbefore defined.

In other aspects the invention further provides:

- a) A method for the treatment or prophylaxis of psoriasis, rheumatoid arthritis or human papilloma virus infection which comprises administering an effective amount of a compound as hereinbefore defined to a mammal;
- b) A method of reducing the toxicity and/or potentiating the efficacy and/or increasing the therapeutic index of 5-FU which comprises administering an

effective amount of a compound as hereinbefore defined to a mammal;

- c) A method of generating 5-ethynyluracil in a mammal which comprises administering a compound as hereinbefore defined wherein the uracil reductase inactivator is 5-ethynyluracil or a prodrug thereof, to a mammal; and
- d) A method of generating 5-fluorouracil in a mammal which comprises administering a compound as hereinbefore defined to a mammal.

Preferably the mammal is a human.

The required dose of the compound may be administered in unit dosage forms. The desired dose is preferably presented as one, two or more sub-doses administered at appropriate intervals. These sub-doses may be administered in unit dosage forms containing for example 1 to 200 mg of the compound.

The compound is preferably administered in the form of a pharmaceutical composition. Thus, in a further aspect the present invention provides a pharmaceutical composition comprising a compound as hereinbefore defined together with at least one pharmaceutically acceptable carrier or excipient, and optionally one or more other therapeutic ingredients.

Each carrier or excipient must be "pharmaceutically acceptable" in the sense of being compatible with the other ingredients of the composition and not injurious to the patient. Compositions include those adapted for oral, rectal, nasal, topical (including buccal, transdermal and sublingual), vaginal and parenteral (including subcutaneous, intramuscular, intravenous and intradermal) administration. The compositions may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. Such methods include the step of bringing into association the active ingredient with the carrier which constitutes one or more accessory ingredients. In general, the compositions are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers or finely divided solid carriers or both, and then if necessary shaping the product.

Compositions of the present invention adapted for oral administration may be presented as discrete units such as capsules, sachets or tablets each containing a predetermined amount of the active ingredient; as a powder or granule; as a solution or a suspension in an aqueous or non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion. The active ingredient may also be presented as a bolus, electuary or paste. Oral administration is the preferred route.

A tablet may be made by compression or moulding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active ingredient in a free flowing form such as a powder or granules, optionally mixed with a binder (e.g. povidone, gelatin, hydroxypropylmethyl cellulose), lubricant, inert diluent, preservative, disintegrant (e.g. sodium starch glycollate, cross-linked povidone, cross-linked sodium carboxymethyl cellulose) surface active or dispersing agent. Moulded tablets may be made by moulding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may optionally be coated or scored and may be formulated so as to provide controlled release of the active ingredient therein using, for example, hydroxypropylmethyl cellulose in varying proportions to provide the desired release profile.

A capsule may be made by filling a loose or compressed powder on an appropriate filling machine, optionally with one or more additives. Examples of suitable additives include binders such as povidone; gelatin, lubricants, inert diluents and disintegrants as for tablets. Capsules may also be formulated to contain pellets or discrete sub-units to provide slow or controlled release of the active ingredient. This can be achieved by extruding and spheronising a wet mixture of the drug plus an extrusion aid (for example microcrystalline cellulose) plus a diluent such as lactose. The spheroids thus produced can be coated with a semi-permeable membrane (for example ethyl cellulose, Eudragit WE30D) to produce sustained release properties.

- 8 -

Compositions for topical administration in the mouth include lozenges comprising the active ingredient in a flavoured base, usually sucrose and acacia or tragacanth; pastilles comprising the active ingredient in an inert base such as gellatin or glycerin, or sucrose and acacia; and mouthwashes comprising the active ingredient in a suitable liquid carrier.

Compositions for rectal administration may be presented as a suppository with a suitable base comprising for example cocoa butter or a salicylate.

Compositions for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams or spray formulations containing in addition to the active ingredient such carriers as are known in the art to be appropriate.

Compositions for parenteral administration include aqueous and non-aqueous isotonic sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the composition isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The compositions may be presented in unit-dose or multidose sealed containers, for example, ampoules and vials, and may be stored in a freeze dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

Preferred unit dosage formulations are those containing a daily dose or unit, daily sub-dose, as hereinabove recited, or an appropriate fraction thereof, of an active ingredient.

Compounds of formula (I) are novel and accordingly a process for the preparation of these compounds provides a further aspect of the present invention.

Compounds of formula (I) may be prepared by reacting a uracil reductase inactivator or prodrug thereof, or a monoprotected derivative of a uracil reductase inactivator or prodrug thereof, with a compound L'-Y wherein Y is as hereinbefore defined or a protected derivative thereof and L' is a group capable of reacting with the uracil

reductase inhibitor to form the linking group L, followed by deprotection where necessary. Suitable protecting groups will be known by one skilled in the art, and include tert-butyldimethylsilyl chloride, trimethylsilylacetylene or p-anisylchloro diphenylmethane(methoxytrityl).

The above coupling reaction may be carried out in a polar aprotic solvent, such as pyridine, in the presence of a coupling reagent, for example dicyclohexylcarbodiimide (DCC) or dimethylaminopyridine (DMAP). The reaction is carried out at a non-extreme temperature of -5°C to 100°C, most suitably room temperature. Methods for the removal of the protecting groups may be carried out by methods known in the art, for example ion exchange using Dowex 50W-X8 [H⁺] in methanol can be used to remove the trityl groups (C. Malange, Chem. Ind. (1987) 856) and silyl protecting groups may be removed using tetraethyl ammonium fluoride in acetonitrile (S. Hanessian et al. Can. J. Chem. (1975) 53, 2975).

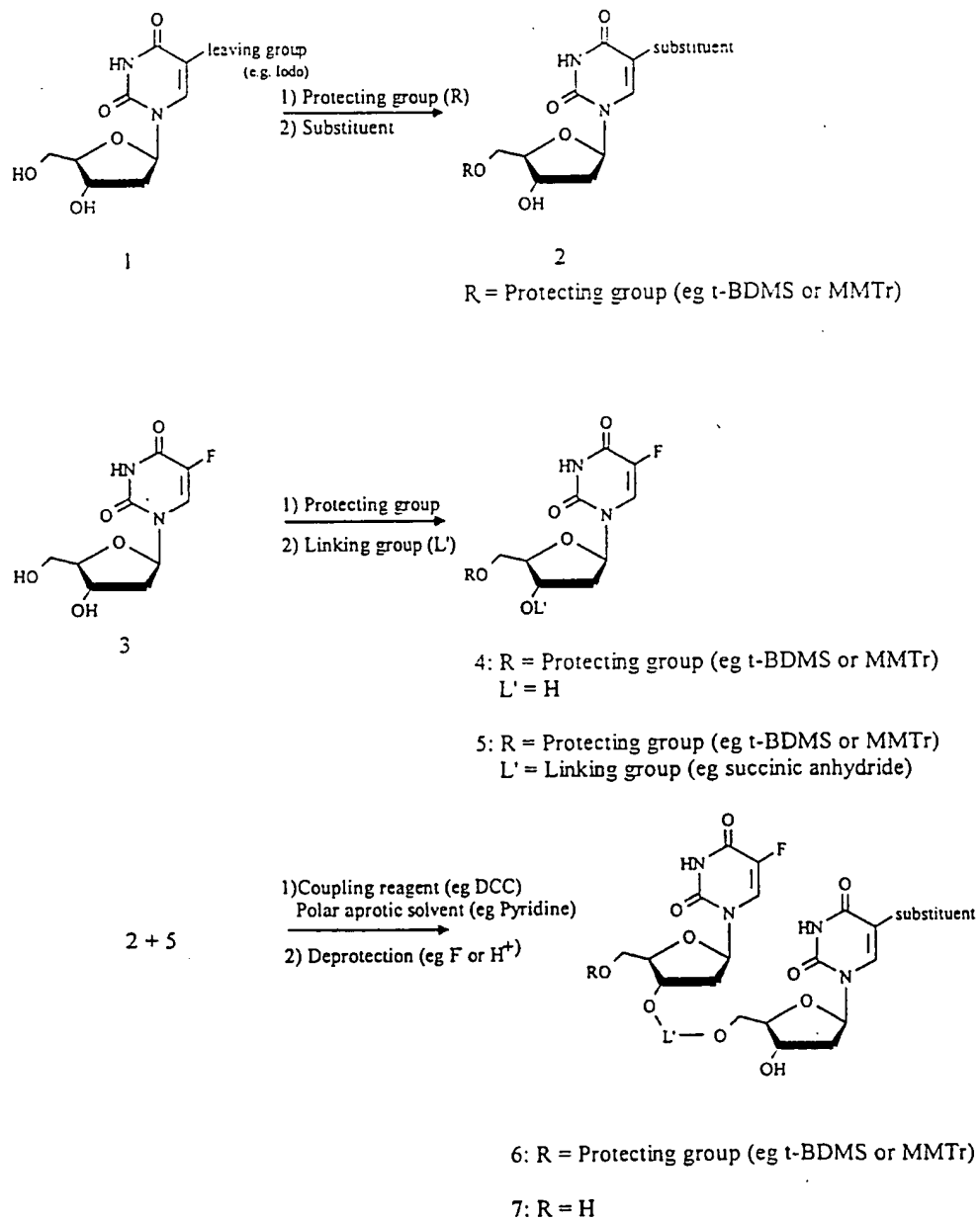
The group L' may be attached to the group Y by the reaction of a compound capable of creating a linking group with the compound Y in the presence of a suitable polar aprotic solvent, for example 1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone (DMTHP). The reaction is carried out at a non-extreme temperature of -25°C to 100°C, most suitably -5°C to room temperature. Compounds capable of forming linking groups include succinic anhydride, phosphorous oxychloride. Such a process for the preparation of a compound of formula (I) wherein X and Y are both nucleoside prodrugs, may more clearly be understood by reference to Scheme 1.

To prepare compounds wherein X and Y are joined via a symmetrical linker, both X and Y may be reacted with a linking group, and the two resulting compounds combined to give a compound of formula (I) as described in Scheme 2 for the preparation of a compound of formula (I) wherein X and Y are both nucleoside prodrugs.

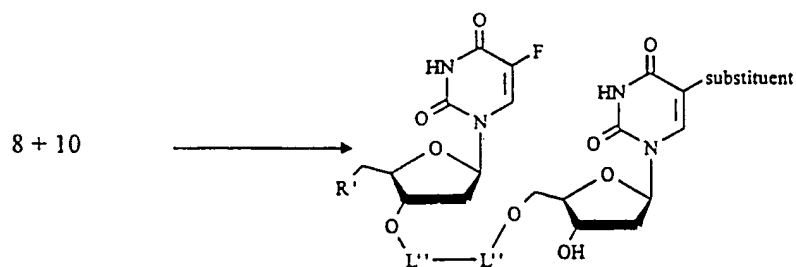
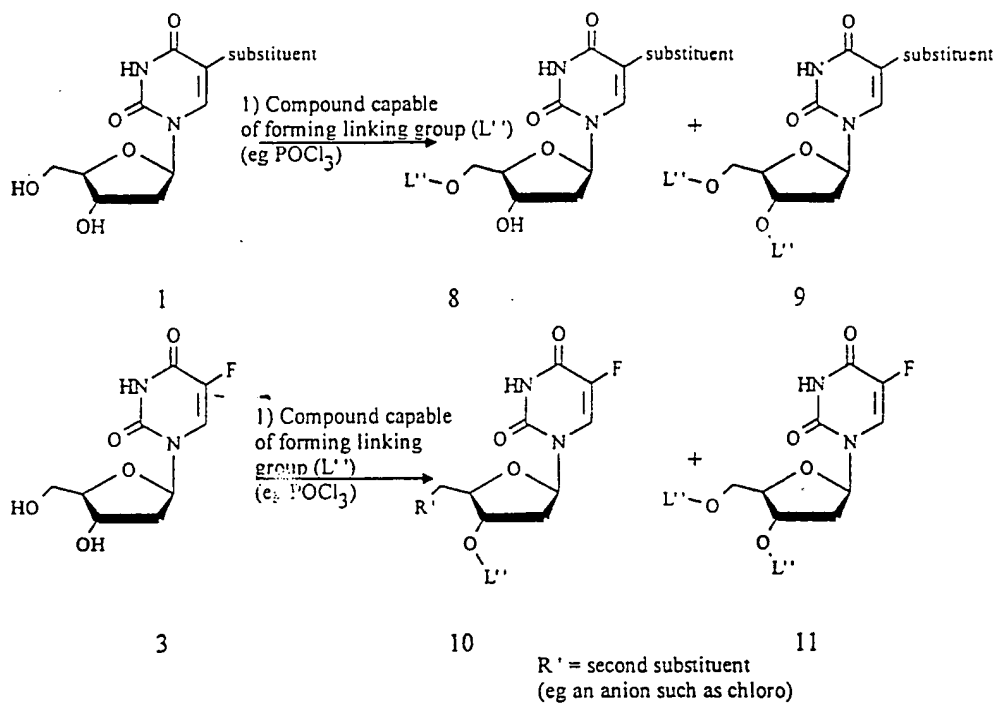
Intermediates of formula L'-Y are novel and accordingly provide a further aspect of the present invention.

- 10 -

Scheme 1 (exemplified using, but not limited to nucleoside prodrugs)



- 11 -

Scheme 2 (exemplified using, but not limited to, nucleoside prodrugs)

The invention will now be described with reference to, but not limited by, the following examples:-

Example 1Preparation of 5-ethynyl-5"-fluoro-3, 3"-O-succinylbis (1-(2-deoxy-β-D-erythropento furanosyl)uracil)

(a) 5'-O-(tert-Butyldimethylsilyl)-2'-deoxy-5-fluorouridine

5-Fluoro-2'-deoxyuridine (United States Biochemical Corporation, Cleveland, Ohio 44120) (6.00g, 24.4 mmol) and 3.65g (5.36 mmol) imidazole (Aldrich Chemical Company, Milwaukee, WI 53233) were dissolved in 25ml anhydrous DMF in a flame dried round bottom flask. 4.0g (26.8 mmol) of tert-Butyldimethylsilyl chloride (Aldrich) were added all at once, the flask was capped with a serum stopper and the solution was magnetically stirred over 3 days. After this time, 100ml of saturated aqueous sodium bicarbonate was poured into the solution and the mixture was extracted with 2x200ml ethyl acetate. The ethyl acetate layer was washed with 200ml water, dried over magnesium sulphate and the solvent was removed on the rotary evaporator. The resulting residue was applied to a column (4.5 X 30cm) packed with silica gel (350g, 230-400 mesh) in dichloromethane. Under medium air pressure (9-12psi) the column was first eluted with 1L of 1% methanol in dichloromethane; successively eluted with 1L of 1.5% methanol in dichloromethane, then 3L of 2% methanol in dichloromethane and finally eluted with 3.5 L of 3% methanol in dichloromethane. Product fractions were pooled and the solvent evaporated to give 6.9 g (19.1 mmol) of the desired product as a white powder; Rf 0.34 (silica, 9:1 chloroform : methanol). ¹H NMR (CDCl₃) δ8.85 (bs, 1H, NH), 8.04 (d, 1H, H6, J_{F,H} = 6.4 Hz), 6.38-6.35 (dd overlapping, 1H, H1'), 4.50-4.46 (m, 1H, H3'), 4.08-4.06 (m, 1H, H4'), 3.97-3.92 (dd, 1H, H5, J = 2.4 Hz, J = 11.5 Hz), 3.86-3.81 (dd, 1H, H5'', J = 2.0 Hz, J = 11.4 Hz) 2.54-2.38 (m, 1H, H2''), 2.18-2.11 (m, 1H, H2'), 0.93 (s, 9H, tert-butyl), 0.15 (s, 6H, silyl methyls).

(b) 5'-O-(tert-Butyldimethylsilyl)-3'-O-(3-Carboxypropionyl)-2'-deoxy-5-fluorouridine

4.0g (11.0mmol) of 5'-O-(tert-Butyldimethylsilyl)-2'-deoxy-5-fluorouridine was dried by twice dissolving the nucleoside in 25ml of anhydrous pyridine in a round bottom flask and evaporating the pyridine on a rotary evaporator. To the

- 13 -

thus dried protected nucleoside re-dissolved in 20ml pyridine was added 0.54g (4.4 mmol) of 4-dimethylaminopyridine all at once and 0.84g (8.4 mmol) succinic anhydride (Aldrich Chemical Company, Milwaukee, WI 53233) in three portions over 90 min. After stirring for 2.5 hrs, another equal portion of 4-dimethylaminopyridine was added. The flask was sealed with a serum stopper and the mixture was allowed to stir overnight. After this time, pyridine was removed on the rotary evaporator by co-evaporation with 4 x 50ml toluene to give a tan foam. The foam was dissolved in 100ml dichloromethane and extracted with 3 x 100ml cold aqueous 10% citric acid, followed by 100ml water. The dichloromethane layer was dried over magnesium sulphate, filtered, and the solvent was removed on a rotary evaporator to give 3.9 g of a tan foam. This foam was dissolved in a minimal amount of dichloromethane and applied to a column (4.5 x 30cm) packed with silica gel (350 g, 230-400 mesh) in dichloromethane. Under medium air pressure (9-12psi) the column was first eluted with 1L of chloroform followed by elution with 1L of 1% methanol in chloroform. The percentage of methanol was increased 1% for each successive litre. The product was eluted into fractions between 2% and 10% methanol in chloroform. Product fractions were pooled and the solvent was removed on the rotary evaporator to give 2.0 g (4.3 mmol) of a white solid: m.p. 133.4-135.3°C.

(c) 5'-O-(tert-Butyldimethylsilyl)-2'-deoxy-5-iodouridine

5-Iodo-2'-deoxyuridine (United States Biochemical Corporation, Cleveland Ohio 44120) (10.0g, 28.2 mmol) and 4.23g (62.1 mmol) of imidazole (Aldrich Chemical Company, Milwaukee, WI 53233) were dissolved in 50ml anhydrous dimethylformamide (DMF) in a flame-dried round bottom flask. 4.7g (31.0 mmol) of tert-Butyldimethylsilylchloride (Aldrich) were added all at once; the flask was capped with a serum stopper and the solution was magnetically stirred for 11 days. After this period of time, the reaction mixture was poured into 300ml aqueous saturated sodium bicarbonate and extracted with 4 x 150ml ethyl acetate. The ethyl acetate extraction's were pooled, dried over magnesium sulphate, filtered and the solvent was removed on a rotary evaporator. The residue was applied to a column (4.5 x 30cm) packed with silica gel (350g, 230-400 mesh) in dichloromethane. Under medium air pressure (9-12psi) the column was first eluted with 2L of dichloromethane,

followed by step-elution with 2L of 1% methanol in dichloromethane, 2L of 2% methanol in dichloromethane and finally 1L of 3% methanol in dichloromethane. Pure product fraction were pooled and solvent removed on a rotary evaporator to give 8.8 g (18.8 mmol) of a white powder: Rf 0.30 (9:1 chloroform: methanol). ^1H NMR (300 MHz, CDCl_3) δ 8.30-8.20 (bs, 1H, NH), 8.10 (s, 1H, H6), 6.32-6.27 (dd overlapping, 1H, H1'), 4.49-4.46 (m, 1H, H3'), 4.10-4.07 (m, 1H, H4'), 3.94-3.89 (dd, 1H, H5'', $J_{5'',5'} = 11.5$ Hz, $J_{5'',4'} = 2.6$ Hz), 3.85-3.81 (dd, 1H, H5', $J_{5',5''} = 11.5$ Hz, $J_{5',4'} = 2.3$ Hz), 2.45-2.42 (m, 1H, H2''), 2.41-2.38 (m, 1H, H2'), 0.96 (s, 9H, tert-butyl), 0.93 (s, 3H, silyl methyl), 0.92 (s, 3H, silyl methyl).

- (d) 1-(5-O-(tert-Butyldimethylsilyl)-2-deoxy- β -D-erythro-pentofuranosyl-5-(2-(trimethylsilyl)ethynyl)uracil)

To a solution of 5.0 g (10.7 mmol) of 5'-O-(tert-butyldimethylsilyl)-2'-deoxy-5-iodouridine, 0.62 g (3.3 mmol) copper (I) iodide, and 3.3 g (4.6 ml, 32.8 mmol) triethylamine in 50 ml dimethylformamide (DMF) under a nitrogen atmosphere was added 4.8 g (7.0 ml, 49.3 mmol) of trimethylsilylacetylene and 1.9 g (1.6 mmol) of tetrakis(triphenylphosphine)palladium(0) (Aldrich Chemical Company, Milwaukee, WI 53233). The dark mixture was magnetically stirred at room temperature overnight. DMF was removed in vacuo on a rotary evaporator and the dark oily residue was dissolved in 100 ml chloroform and extracted with 2 x 150 ml of aqueous 5% ethylenediamine tetra acetic acid disodium salt, followed by 1 X 150 ml water. The chloroform layer was dried over magnesium sulphate, filtered and the solvent removed on a rotary evaporator. The residue was dissolved in a minimal amount of chloroform and applied to a column (4.5 x 30 cm) packed with silica gel (350g, 230-400 mesh) in chloroform. Under medium air pressure (9-12 psi) the column was eluted with 2L of chloroform followed by 3.5L of 1% methanol in chloroform to elute the desired product. After removal of the solvent on a rotary evaporator, 2.7 g (6.2 mmol) of product was obtained as a tan solid: Rf 0.51 (silica, 9:1 chloroform: methanol). ^1H NMR (300 MHz, CDCl_3) δ 8.15-8.10 (bs, 1H, NH), 7.97 (s, 1H, H6), 6.34-6.29 (dd overlapping, 1H, H1'), 4.51-4.48 (m, 1H, H3') 4.10-4.08 (m, 1H, H4'), 3.95-3.90 (dd, 1H, H5'', $J_{5'',5'} = 11.4$ Hz, $J_{5'',4'} = 2.6$ Hz), 3.85-3.80 (dd 1H, H5', $J_{5',5''} = 11.4$ Hz, $J_{5',4'} = 2.1$ Hz), 2.41-

- 15 -

2.37 (m, 1H H^{2''}), 2.16-2.11 (m, 1H H^{2''}), 1.86 (s, 9H, C(CH₃)₃), 0.20 (s, 9H, acetylenic Si(CH₃)₃), 0.15 (s, 3, Silyl methyl), 0.13 (s, 3H, silylmethyl).

- (e) 5-Fluoro-5''-(2-(trimethylsilyl)ethynyl-3',3'''-O-succinylbis-(1-(2-deoxy-5-O-(tert-butylidimethylsilyl)-β-D-erythro-pentofuranosyl)uracil)

In a previously flame dried round bottom flask was dissolved 0.51g (1.1 mmol) of 1-(5-O-(tert-butylidimethylsilyl)-2-deoxy-β-D-erythro-pentofuranosyl-5-(2-(trimethylsilyl)ethynyl)uracil and 0.49g (1.1mmol) of 5'-O-(tert-butylidimethylsilyl)-3'-O-(3'-carboxypropionyl)-2'-deoxy-5-fluorouridine in 5ml anhydrous pyridine. To this solution was added 0.46g (2.2mmol) of dicyclohexylcarbodiimide (Aldrich Chemical Company, Milwaukee, WI 53233) and the solution was allowed to magnetically stir overnight. The following day, pyridine was removed on the rotary evaporator and the orange-brown syrup was resuspended in ice-cold ethyl acetate. Dicyclohexylurea was removed by vacuum filtration and the ethyl acetate was subsequently removed on the rotary evaporator. The residue was re-dissolved in a minimal amount of dichloromethane and applied to a column (4.5 x 30 cm) packed with silica gel (350 g, 230-400 mesh) in dichloromethane. Under medium air pressure (9-12 psi) the column was eluted with 2L of dichloromethane followed by 4L of 1.5% methanol in dichloromethane for elution of an impure product. The product was re-chromatographed across 100g silica packed in hexanes under medium air pressure and eluted by an increasing step-gradient from 5 to 50% ethyl acetate in hexanes. After removal of solvent on a rotary evaporator, 0.60g (0.68 mmol) of the product was obtained as a white powder: R_f 0.54 (silica, 9:1 chloroform: methanol); m.p. 139.8°C, resolidifies to melt at 208-210°C.

- (f) 5-Ethynyl-5''-fluoro-3',3''-O-succinylbis-(1-(2-deoxy-β-D-erythro-pentofuranosyl)uracil)

0.90g (1.0mmol) of 5-Fluoro-5''-(2-(trimethylsilyl)ethynyl-3',3'''-O-succinylbis (1-(2-deoxy-5-O-(tert-butylidimethylsilyl)-beta-D-erythro-pentofuranosyl)uracil) was treated with 3.3 equivalents of tetraethyl ammonium fluoride hydrate (Aldrich Chemical Company, Milwaukee, WI 53233) in 25ml acetonitrile and allowed to magnetically stir at ambient temperature overnight.

- 16 -

The following day, an additional equivalent of the fluoride reagent was added and the solution was allowed to stir for several more hours. The solution was then directly applied to a 100g silica gel (230-400 mesh) column (2.5 x 30 cm) packed in dichloromethane. The column was eluted with 500ml of dichloromethane, followed by a step-gradient of 1 to 5% methanol in dichloromethane. Overnight, the fractions precipitated out of the 5% methanol fractions to give 156mg of analytically pure product after filtration and drying. The combined mother liquors were combined and evaporated to give an additional 270mg of product after drying for a total of 0.426g (0.73 mmol): m.p. 229.5-230.6 °C. ¹H NMR (DMSO-d₆) δ 11.9-11.6 (bs, 2H, N3H & N3"H), 8.29 (s, 1H, H6), 8.21 (d, 1H, H6", J_F, H = 7.1 Hz), 6.16-6.12 (m, 2H, H1' & H1""), 5.33-5.30 (m, 2H, H3' & H3""), 5.29-5.24 (m, 2H, 5'OH & 5""OH), 4.14 (s, 1H, acetylenic H), 4.04-4.01 (m, 2H, H4' & H4""), 3.65-3.63 (m, 4H<H5' & H5""), 2.65 (s, 4H, succinate), 2.33-2.26(m, 4H, H2' & H2"").

Example 2

Preparation of 5-ethynyl-5"-fluoro-3',3"-O-succinylbis(1-(2-deoxy-β-D-erythropento furanosyl)uracil)

(a) 2'-Deoxy-5-fluoro-5'-O-(4-methoxytrityl)uridine

5-Fluoro-2'-deoxyuridine (United States Biochemical Corporation, Cleveland, Ohio 44120) (1.0g, 4.1 mmol) was twice suspended in 30ml anhydrous pyridine in a round bottom flask and the pyridine removed on a rotary evaporator at 50° C and oil pump vacuum. The deoxyribonucleoside thus dried was re-dissolved in 41 ml pyridine and cooled to ca. 5°C in an ice-water bath. The solution was magnetically stirred. To the solution was added 0.06 equivalents (30mg, 0.24 mmol) 4-dimethylaminopyridine and 1.4 equivalents triethylamine (0.8 ml, 5.7 mmol) all at once. This was followed by the addition of 1.2 equivalents (1.5g, 4.9 mmol) p-anisylchlorodiphenylmethane (Aldrich Chemical Company, Milwaukee, WI 53233) in one-quarter portions every 45 min. After the final addition of p-anisylchlorodiphenylmethane, the ice-bath was allowed to gradually warm to ambient temperature. The reaction was sealed with a serum stopper and magnetically stirred overnight. After this period an additional 350 mg of p-anisylchlorodiphenylmethane was added and the reaction was allowed

- 17 -

to stir for another 8 hrs. The reaction was then poured into 100 ml water in a separating funnel and extracted with 4 x 100 ml diethyl ether. Diethyl ether was removed on a rotary evaporator. 2.4 g of crude material was dissolved in a minimal amount of dichloromethane and applied to a column (2.5 x 30 cm) packed with silica gel (100 g, 230 - 400 mesh) in dichloromethane. Under medium air pressure (9 - 12 psi) the column was first eluted with 2L of dichloromethane followed by 1L of 3% methanol in dichloromethane to elute the product. After evaporation of solvent, the product was obtained as a white foam (1.8 g, 3.2 mmol): Rf 0.42 (silica, 9:1 chloroform: methanol). ¹H NMR (300 MHz, DMSO-d₆) δ 11.87 (s, 1H, NH) 7.88 (d, 1H, H₆, J_F, H = 6.85 Hz), 7.42-7.23 (m, 10H, Ar H), 6.90 (d, 2H, Ar H, J = 8.89Hz), 6.14-6.12 (overlapping dd, 1H, H1'), 5.34 (d, 1H, 3' OH, J = 4.57Hz), 4.27 (m, 1H, H3'), 3.88 (m, 1H, H4') 3.75 (s, 3H, OCH₃) 3.34-3.25 (m, 1H, H5'), 3.16-3.12 (m, 1H, H5''), 2.26-2.16 (m, 2H, H2'' & H2').

(b) 2'-Deoxy-5-iodo-5'-O-(4-Methoxytrityl)uridine

5-Iodo-2'-deoxyuridine (United States Biochemical Corporation, Cleveland, Ohio 44120) (1.0 g, 2.8 mmol) was twice suspended in 30 ml anhydrous pyridine in a round bottom flask and the pyridine removed on a rotary evaporator at 50°C and oil pump vacuum. The deoxynucleoside thus dried was re-dissolved in 28 ml pyridine and cooled to ca. 5°C in an ice-water bath. The solution was magnetically stirred. To the solution was added 0.06 equivalents (20 mg, 0.16 mmol) 4 -dimethylaminopyridine and 1.4 equivalents (0.55 ml, 4.0 mmol) triethylamine all at once followed by the addition of 1.2 equivalents (1.0g, 3.4 mmol) of p-anisylchlorodiphenylmethane (Aldrich Chemical Company, Milwaukee, WI 53233) in one-quarter portions every 45 min. After the addition of the final portion of p-anisylchlorodiphenylmethane, the ice bath was allowed to gradually warm to ambient temperature. The reaction was sealed with a serum stopper and magnetically stirred overnight. After this period, an additional 250 mg of p-anisylchlorodiphenyl methane was added and the reaction was allowed to stir for an additional 8 hrs. The reaction was then poured into 100ml water in a separating funnel and extracted with 4 x 100 ml diethyl ether. The diethyl ether extracts were cooled and the solvent was removed on a rotary evaporator. 2.4g of crude material was dissolved in a

minimal amount of dichloromethane and applied to a column (2.5 x 30 cm) packed with silica gel (100g, 230-400 mesh in dichloromethane). Under medium air pressure (9-12 psi), the column was first eluted with 1L of dichloromethane, then with 1L of 1% methanol in dichloromethane and finally with 1L of 5% methanol in dichloromethane to elute the product. After evaporation of solvent, the product was obtained as an off-white foam (1.3 g, 1.9 mmol): R_f 0.48 (silica, 9:1 chloroform : methanol). 1H NMR (300 MHz, DMSO- d_6) δ 11.75 (s, 1H, NH), 8.02 (s, 1H, H6), 7.43-7.23 (m, 10H, Ar H), 6.91 (d, 2H, Ar H, J = 8.89 Hz), 6.13-6.08 (overlapping dd, 1H, H1'), 5.32 (d, 1H, 3' OH, J = 4.57 Hz) 4.24 (m, 1H, H3'), 3.91 (m, 1H, H4'), 3.75 (s, 3H, OCH₃), 3.34-3.17 (m, 2H, H5' & H5''), 2.26-2.17 (m, 2H, H2' & H2).

(c) 2'-Deoxy-5-ethynyl-5'-O-(4-methoxytrityl)uridine

2'-Deoxy-5-iodo-5'-O-(4-methoxytrityl)uridine (1.2g, 1.8 mmol) was dissolved in 6ml of anhydrous N,N-dimethylformamide (DMF) and the solution was vigorously deoxygenated with nitrogen for 30 min. To the magnetically stirred solution was added 142mg (0.20mmol) bis(triphenylphosphine)palladium(II) chloride, 76mg (0.40 mmol) copper (I) iodide, 0.53ml (5.40 mmol) of trimethylsilylacetylene and 0.56ml (4.0 mmol) of triethylamine (Aldrich Chemical Company, Milwaukee, WI 53233). The following day identical portions of bis(triphenylphosphine)palladium(II)chloride, copper (I) iodide, trimethylsilylacetylene and triethylamine were added. The reaction was allowed to stir for an additional 5 hrs before being poured into 100ml ethyl acetate in a separating funnel. The ethyl acetate solution was extracted with 3 x 30ml aqueous 5% ethylenediamine tetra acetic acid disodium salt and washed with 1 x 30ml water. Ethyl acetate was removed on the rotary evaporator to give a black foam. This foam was dissolved in 5ml of acetonitrile and 222mg (1.5 mmol) of tetraethyl ammonium fluoride hydrate (Aldrich Chemical Company, Milwaukee, WI 53233) was added and the solution was magnetically stirred for 1 hr. After this period the reaction mixture was loaded directly onto a column (2.5 C 30cm) packed with silica gel (100g, 230-400 mesh) in chloroform. Under medium air pressure (9-12 psi) the column was first eluted with 1L of chloroform, then 1 L of 1% methanol in chloroform and finally with 1L of 2.5 % methanol in chloroform to elute the product. The appropriate

product fractions were combined and solvent was removed on a rotary evaporator to 769mg (1.5 mmol) of a light brown foam: Rf 0.23 (silica, 95:5 chloroform : methanol) ; IR (1% KBr) acetylenic carbon-carbon stretch 2110 cm^{-1} ; MS (EI, Cl₂) 524 (M). ¹H NMR (300 MHz, DMSO - d₆) δ 11.69 (s, 1H, NH), 7.94 (s, 1H, H₆), 7.41-7.21 (m, 10H, Ar H), 6.90 (d, 2H, Ar H, J = 8.9 Hz), 6.11-6.07 (overlapping dd, 1H, H_{1'}), 5.30 (d, 1H, 3' OH, J = 4.6 Hz), 4.24 (m, 1H, H_{4'}), 3.74 (s, 3H OCH₃), 3.41-3.11 (m, overlapping water peak, H_{5'} & H_{5''}), 2.27-2.18 (m, 2H, H_{2''} & H_{2'''}).

- (d) 5-Ethynyl-5''-fluoro-3',3'''-O-succinylbis(1-(2-deoxy-5-O-(4-methoxytrityl)- β -D-erythro-pentofuranosyl)uracil)

To a 50ml flame dried round bottom flask with magnetic stir bar was added 726mg (1.3 mmol) of 2'-deoxy-5-fluoro-5'-O-(4-methoxytrityl)uridine, 32mg (0.26mmol) of 4-dimethylaminopyridine (DMAP) and 143mg (1.4mmol) of succinic anhydride. The mixture was dissolved in 6ml of pyridine sealed under a septum and magnetically stirred overnight. The following day an additional 30mg of DMAP and 30mg of succinic anhydride was added and the reaction was stirred for an additional 24h. On the third day, 1.05g (5.1mmol) of 2'-deoxy-5-ethynyl-5'-O-(4-methoxytrityl)uridine was added and the mixture was allowed to stir overnight. The following day, dicyclohexylurea was removed via suction filtration through a sintered glass funnel and was washed with ethyl acetate. The filtrate was partitioned between ethyl acetate and water. The ethyl acetate layer was dried over magnesium sulphate, filtered and evaporated on a rotary evaporator. The title compound was obtained by successive medium air pressure (9-12 psi) silica gel (100g, 230-400 mesh) column (2.5 x 30 cm) chromatography eluting with dichloromethane, followed by 1% then 3% methanol in chloroform. After evaporation of solvent, the product was obtained as a white foam (0.27g, 0.22 mmol): Rf 0.35 (silica, 9:1 chloroform : methanol). ¹H NMR (300 MHz, DMSO-d₆) δ 12.00-11.68 (2 bs 2H, N₃H & N_{3''}H), 7.97 (s, 1H, H₆), 7.89 (d, 1H, H_{6''}, J_{F,H} = 6.8 Hz), 7.40-7.20 (m, 10H, Ar H), 6.88 (d, 2H, Ar H, J=8.8Hz), 6.18-6.10 (m, 2H, H_{1'} & H_{1'''}) 5.25-5.23 (m, 2H, H_{3'} & H_{3'''}), 4.07 (m, 2H, H_{4'} & H_{4'''}), 4.00 (s, 6H, 2 X OCH₃), 3.20-3.40 (m, obscured by water, H_{5'} & H_{5'''}) 2.40 (s, 4H, succinate), 2.50-2.20 (m, 4H, H_{2'} & H_{2'''}).

- (e) 5-Ethynyl-5"-fluoro-3',3'''-O-succinylbis(1-(2-deoxy-β-D-erythro-pentofuranosyl)uracil)

0.130g (0.11mmol) of 5-Ethynyl-5"-fluoro-3',3'''-O-succinylbis(1-(2-deoxy-5-O-(4-methoxytrityl)-β-D-erythropentofuranosyl)uracil) was dissolved in 10ml of anhydrous methanol in a round bottom flask. 400mg of methanol-washed Dowex-50WX8 (H⁺ form, 20-50 mesh, J.T. Baker) was added and the mixture warmed to 55°C for 4h. The solution was filtered to remove the resin and the beads washed with methanol. The filtrate was rotary evaporated to dryness. The off-white solid was suspended in dichloromethane and collected by suction-filtration, washed with dichloromethane then water and dried in a vacuum oven overnight at 50°C. 0.035g (0.059 mmol) of an off-white solid was obtained. ¹H NMR (300 MHz, DMSO-d₆) δ 12.0 (bs, 2H, N3H & N3"H), 8.29 (s, 1H, H6), 8.21 (d, 1H, H"6, J_F, H = 7.1 Hz), 6.16-6.12 (m, 2H, H1' & H1""), 5.35-5.30 (m, 2H, H3' & H3""), 5.59-5.24 (m, 2H, 5'OH & 5""OH), 4.14 (s, 1H, acetylenic H), 4.04-4.01 (m, 2H, H4' & H4""), 3.65-3.63 (m, 4H, H5' & H5""), 2.65 (s, 4H, succinate), 2.33-2.26 (m, 4H, H2' & H2"").

Example 3

Preparation of 5'-chloro-2',5'-Dideoxy-5-Dideoxy-5-fluorouridine 2'''-Deoxy-5"-Ethynyluridine(3'-5''') Diphosphate

- (a) 2'-Deoxy-5-(2-(Trimethylsilyl)Ethynyl)Uridine

The title compound was prepared according to the procedure of Morris J. Robbins and Philip J Barr, *J.Org. Chem.*, 1983, 48, 1854-1862.

A mixture of 5-iodo-2'-deoxyuridine (U.S. Biochemical Corp., Cleveland, Ohio) (2.7mmol), dimethylformamide (8ml), and triethylamine (0.6ml) was deoxygenated with a rapid stream of N₂ over 10 min in a flame dried 100ml round-bottom flask with magnetic stirring. To this deoxygenated solution was added copper iodide (Aldrich Chemical Co., Milwaukee, WI) (1.0mmol), bis(triphenylphosphine)palladium(II)chloride (Aldrich) (0.5mmol), and (trimethyl silyl)acetylene (Aldrich) (5.4mmol). The mixture was allowed to

magnetically stir at room temperature overnight, at which time there was no starting nucleoside as evidenced by TLC (Silica, 9:1 chloroform: methanol). DMF was removed on a vacuum pump at ambient temperature. The crude material was applied in chloroform to a 70g silica gel (230-400 mesh) column (2.5 x 30cm) packed in 3% methanol in chloroform. Under medium air pressure (9-12 psi), the column was eluted with 1L of 3% methanol in chloroform, and then 1L of 5% methanol in chloroform. 100 ml fractions were collected. Fractions 10-15 contained the desired product. The product fractions were pooled and solvent was removed on a rotary evaporator to give the product as a hygroscopic yellow foam (2.3 mmol, 85% yield): R_f 0.15 (silica, 9:1 chloroform: methanol). IR (KBr) 2167 cm^{-1} , acetylenic C \equiv C stretch. ^1H NMR (300 MHz, DMSO- d_6) δ 11.7-11.6 (bs, 1H, N-H), 8.26 (s, 1H, H-6), 6.11-6.07 (t, 1H, H1', $J=6.5$ Hz), 5.26-5.24 (d, 1H, 3'-OH, $J=4.3$ Hz), 5.13-5.09 (t, 1H, 5'-OH, $J=5.0$ Hz), 4.25-4.20 (m - 5 Lines, 1H, H3'), 3.80-3.76 (q, 1H, H4'), 3.63-3.55 (m, 2H, H5" and H5' overlapping), 2.16-2.10 (m, 2H, H2" and H2'), 0.20 (s, 9H, Si(CH $_3$) $_3$).

(b) 2'-Deoxy-5-Ethynyluridine

The title compound was prepared according to a modification of the procedure of Morris J. Robbins and Philip J Barr, J. Org. Chem., 1983, **48**, 1854-1862.

A mixture of 2'-deoxy-5-(2-(trimethylsilyl)ethynyl)uridine (2.4 mmol) and tetraethyl ammonium fluoride hydrate (Aldrich Chemical Co., Milwaukee, WI) (2.4 mmol) was magnetically stirred in 10 ml dry acetonitrile (Aldrich) for 1 hour. The solution was applied directly to a column (2.5 x 30 cm) of 100g silica gel (230-400 mesh) packed in dichloromethane. The column was eluted with 3% methanol in dichloromethane to obtain pure product (1.7 mmol, 71% yield) as an off-white solid after evaporation of solvent. IR (KBr) 2120 cm^{-1} , C \equiv C stretch. ^1H NMR (300 MHz, DMSO- d_6) δ 11.58-11.65 (bs, 1H, NH), 8.30 (s, 1H, H6), 6.13-6.08 (t, 1H, H1', $J=6.55$ Hz), 5.26-5.24 (d, 1H, 3'-OH, $J=4.3$ Hz), 5.15-5.12 (t, 1H, 5'-OH, $J=4.90$ Hz), 4.26-4.21 (m - 5 lines, 1H, H3'), 4.11 (s, 1H, acetylenic H), 3.82-3.79 (q, 1H, H4'), 3.63-3.56 (m, 2H, H5" and H5'), 2.15-2.12 (m, 2H, H2" and H2').

- 22 -

- (c) 1-(2-Deoxy- β -D-Erythro-Pentofuranosyl)-5-Ethynyluracil 5'-Monophosphate and 1-(2-Deoxy- β -D-Erythro-Pentofuranosyl)-5-Ethynyluracil 3',5'-Diphosphate

2'-Deoxy-5-ethynyluridine (1.7 mmol) was co-evaporated several times in a 25 ml round-bottom flask with dry acetonitrile (Aldrich Chemical Co., Milwaukee, WI). The nucleoside was dissolved in 5ml of 1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone (Aldrich) with magnetic stirring. The flask was sealed with a serum stopper and the solution stirred in a methanol-ice bath at -12°C for 5 minutes. 3 equivalents (0.4ml) of phosphorous oxychloride (Aldrich) from a freshly opened ampoule was added to the nucleoside solution. After 5 min reaction time, the mixture was quenched with 5ml of water with continued stirring. After 3 min, 0.5ml of tributylamine (Aldrich) was added. The product mixture was diluted with water and loaded onto 80g of DEAE Sephadex swollen in 50mM ammonium bicarbonate. The column was eluted with an increasing gradient of aqueous ammonium bicarbonate (50mM to 500mM) with UV detection at 280nm. Elution flowrate was as controlled by Gilson Minipuls 2 peristaltic pumps (Gilson, Middleton, WI). The first eluting product fractions were pooled and evaporated on a rotary evaporator at 45°C. The resulting white solid was co-evaporated multiple times with water on the rotary evaporator, then re-dissolved in 15ml of water, frozen and lyophilised to give 0.34mmol (20% yield) of the 5'-monophosphate as a white monoammonium salt. HPLC was performed on a 4.6mm X 100mm, 5 micron, analytical strong anion exchange column (Alltech, Deerfield, IL) with a linear gradient of 10mM to 1M ammonium phosphate, pH 5.5, 5% methanol over 30 min. This product had a retention time (R_t) of 3.4 minutes. Negative Ion FAB MS (dithioerythritol/dithiothreitol) ($M-H$)⁻ = 331.1. ³¹P NMR (121.421 MHz, ¹H decoupled, 85% phosphoric acid δ = 0.0, DMSO-d₆) δ 0.32 (s, 1P, 5' phosphate). ¹H NMR (300 MHz₂, DMSO-d₆) δ 7.97 (s, 1H, H6), 6.09-6.05 (overlapping dd, 1H, H1'), 4.27-4.25 (bs, 1H, H3'), 4.05 (s, 1H, acetylenic H) 3.89-3.85 (bs, 1H, H4'), 3.79-3.76 (m, 2H, H5' and H5'') 2.15-2.05 (H2' and H2'').

A second product eluted after the 5'-monophosphate. Identical fractions were pooled, evaporated and lyophilised as before to give 0.62 mmol (37% yield) of

the 3',5'-diphosphate of the starting nucleoside. Analytical HPLC (as above), R_t = 8.0 min. Negative Ion FAB MS (dithioerythritol/dithiothreitol) $(M-H)^- = 411.0$. ^{31}P NMR (121.421 MHz, 1H decoupled, 85% phosphoric acid $\delta = 0.0$, DMSO- d_6) $\delta -0.01$ (s, 1P, 5' phosphate), -1.07 (s, 1P, 3' phosphate). 1H NMR (300 MHz, DMSO- d_6) $\delta 8.00$ (s, 1H, H6), 6.10-6.05 (t, 1H, H1', $J=7.1$ Hz), 4.76-4.74 (m - broad, 1H, H3'), 4.17-4.16 (s, 1H, H4'), 4.06 (s, 1H, acetylenic H), 3.90-3.78 (2 multiplets, 2H, H5'' and H5'), 2.22-2.21 (m - broad, 2H, H2'' and H2').

The 5'-monophosphate and the 3',5'-diphosphate were assayed for ammonia according to the assay of A. L. Chaney and E. P. Marbach, Clin. Chem. 1962, **8**, 130-132. The phosphates were assayed for phosphorous according to procedure of B.N. Ames, Methods Enzymol. 1966, **8**, 115-118.

- (d) Preparation of 1-(2-Deoxy- β -D-Erythro-Pentofuranosyl)-5-Ethynyluracil 5'-Monophosphate From 1-(2-Deoxy- β -D-Erythro-Pentofuranosyl)-5-Ethynyluracil 3',5'-Diphosphate By Enzymatic Dephosphorylation With Nuclease P1

To 1-(2-deoxy- β -D-erythro-pentofuranosyl)-5-ethynyluracil 3',5'-diphosphate (0.50 mmoles) in 5 ml of water was added 50 microliters of aqueous 1M $ZnCl_2$ and 250 microliters of a 30 mM sodium acetate buffer, pH 5.0, containing 1 unit of Nuclease P1 (from *Penicillium citrinum*, Boehringer Mannheim, Indianapolis, IN) per microliter. The mixture was heated in a water bath at 50 ° C for 3 hours. The product was purified and characterised as previously described. The yield was quantitative.

- (e) 5'-Chloro-2',5'-Dideoxy-5-Fluorouridine 3'-O-Phosphate and 2'-Deoxy-5-Fluorouridine 3',5'-Di-O-Phosphate

This is a modification of the procedure found in European Patent Application No. 910502.6; Inventor, S.M. Tisdale et al.; Title, Antiviral Compounds. and Bull.Chem.Soc.Japan (1969), **42**, 350.

2'-Deoxy-5-fluorouridine (4.1mmol) (United States Biochemical, Cleveland, OH) was dissolved in 7ml of 1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-

pyrimidinone (Aldrich) in a vial equipped with a magnetic stir bar and cap. The vial was cooled to -5°C in an ice-methanol bath. 1.5ml of phosphorous oxychloride was added to the stirring solution and the solution continued to stir in the bath for 7 min. The reaction was then quenched by the addition of 5ml of water, and the mixture was allowed to stir in the cold bath for 5 min. The mixture was poured into 30 ml of tributylamine, diluted with 900 ml water and loaded onto 80g of DEAE Sephadex swollen in 50 mM ammonium bicarbonate. The column was eluted with an increasing gradient of aqueous ammonium bicarbonate (50 mM to 500 mM) with UV detection at 267 nm. Elution flowrate was controlled by Gilson Minipuls 2 peristaltic pumps (Gilson, Middleton, WI). The first eluting product fractions were pooled and evaporated on a rotary evaporator at 45°C . The resulting white solid was co-evaporated multiple times with water on the rotary evaporator, then re-dissolved in 15ml of water, frozen and lyophilised to give 1.2 mmol (29% yield) of the 5'-chloro-2',5'-dideoxy-5-fluorouridine 3'-O-phosphate as the mono-ammonium salt. HPLC was performed on a 4.6 mm x 100mm, 5 micron, analytical strong anion exchange column (Alltech, Deerfield, IL) with a linear gradient of 10mM to 1M ammonium phosphate, pH 5.5, 5% methanol over 30 min. This product had a retention (R_t) of 3.6 minutes. Positive Ion FAB MS (glycerol) $(M+1)^+ = 345.1$. ^{31}P NMR (121.421 MHz, ^1H decoupled, 85% phosphoric acid $\delta = 0.0$, DMSO- d_6) δ -0.56 (s, 1P, 3'-phosphate). ^1H NMR (300 MHz, DMSO- d_6) δ 7.96-7.94 (d, 1H, H6, $J = 7.0$ Hz), 6.15-6.13 (triplet - broadened, 1H, H1'), 4.56-4.54 (bs, 1H, H3'), 4.17-4.16 (bs, 1H, H4'), 3.95-3.90 (overlapping multiplets, 2H, H5" and H5', 2.39-2.19 (m, 2H, H2" and H2').

A second product eluted after the 5'-monophosphate. Identical fractions were pooled, evaporated and lyophilised as before to give 0.62 mmol (37% yield) of the 3',5'-diphosphate of the starting nucleoside. Analytical HPLC (as above), $R_t = 8.5$ min. Positive Ion FAB MS (glycerol) $(M+1)^+ = 407.4$. ^{31}P NMR (121.421 MHz, ^1H decoupled, 85% phosphoric acid $\delta = 0.0$, DMSO- d_6) δ -0.369 (s, 1P, 5' phosphate), -1.45 (s, (1P, 3' phosphate). ^1H NMR (300 MHz, DMSO- d_6) δ 8.12-8.10 (d, 1H, H6, $J = 7.0$ Hz), 6.12-6.10 (t, 1H, H1'), 4.82-4.79 (m, 1H, H3'), 2.23-2.20 (m, 2H, H2" and H2').

The phosphates were assayed for phosphorous according to procedure of B.N.

Ames, Methods Enzymol. 1966, 8, 115-118.

- (f) 5'-Chloro-2',5'-Dideoxy-5-Fluorouridine 2'''-Deoxy-5''-Ethynyluridine (5',5''')
Diphosphate

This phosphoimidazolidate procedure is based on the synthesis of triphosphates from monophosphates in the paper by Donald E. Hoard and Donald G. Ott, J. Am. Chem. Soc. 1965, 87, 1785-1788.

The 5' monophosphate of 1-(2-deoxy- β -D-erythro-pentofuranosyl)-5-ethynyl uracil (0.61 mmol) was co-evaporated with 2 x 50ml 0.5M triethylammonium bicarbonate at 40°C on a rotary evaporator to a tan foam. The nucleotide was then co-evaporated with 3 x 75ml of dry acetonitrile (Aldrich Chemical Co., Milwaukee, WI) in the same round-bottom flask. The nucleotide thus dried was dissolved in 5ml of 1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone (Aldrich) with magnetic stirring. Approximately 5 equivalents (498 mg) of 1,1'-carbonyldiimidazole was added to the solution and the mixture stirred for 1h with the flask sealed with a serum stopper. At this time, 157 microliters of methanol was added and the solution stirred for 75 min to destroy excess 1,1'-carbonyldiimidazole.

1.2 Equivalents of 5'-chloro-2',5'-dideoxy-5-fluorouridine 3'-O-phosphate was co-evaporated with 0.5M triethylammonium bicarbonate, then dry acetonitrile as the previous nucleoside. The 5-fluoro nucleotide was then dissolved in 4ml of 1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone and added to the 5-ethynyl nucleotide phosphorimidazolidate mixture. This new mixture was allowed to stir overnight. The following day an additional 0.25 equivalent of the 5-fluoro nucleotide, similarly prepared, was added to the phosphorimidazolidate mixture and the resulting solution stirred over the weekend. This condensation mixture was then diluted into 150ml aqueous ammonium hydroxide, pH 9.5; 500 microliters of alkaline phosphatase (Boehringer Mannheim, Indianapolis, IN) was added to cleave non-coupled nucleotides and the mixture was allowed to stir overnight. The following day, the alkaline solution was diluted to 600ml total volume with water and loaded onto 80g of DEAE Sephadex swollen in 50 mM ammonium bicarbonate. The

- 26 -

column was eluted with an increasing gradient of aqueous ammonium bicarbonate (50 mM to 500 mM) with UV detection at 270 nm. Elution flowrate was controlled by Gilson Minipuls 2 peristaltic pumps (Gilson, Middleton, WI). Fractions were analysed by HPLC with a 4.6 mm x 100 mm, 5 micron, analytical strong anion exchange column (Alltech, Deerfield, IL) and a linear gradient of 10 mM to 1 M ammonium phosphate, pH 2.4, 5% methanol over 30 min. Pure product fractions were pooled and water was removed on a rotary evaporator. The resulting white solid was co-evaporated multiple times with water on the rotary evaporator, then re-dissolved in 15 ml of water, frozen and lyophilised to give 0.18 mmoles (30% yield) of the title product. Analytical HPLC (as above), $R_t = 5.62$ min. Negative Ion FAB MS (dithioerythritol/dithiothreitol) $(M-H)^- = 657.0$. ^{31}P NMR (1 MHz, ^1H decoupled, 85% phosphoric acid $\delta = 0.0$, D_2O) δ (-) 10.14 - (-) 10.27 (d, 1P, 5'-Phosphate, $J_{\text{P,P}} = 21.48$ Hz), (-) 10.94 - (-) 11.08 (d, 1P, 3'-Phosphate, $J = 21.48$ Hz) (^{31}P assignments made by $^1\text{H} - ^{31}\text{P}$ HMQC NMR pulse sequence). ^1H NMR (400 MHz, D_2O) δ 8.05 (s, 1H, H6"), 7.80 (d, 1H, H-5, $J = 7.0$ Hz) 6.17-6.15 (m - overlapping triplets, 2H, H1' and H1"), 4.78-4.74 (m, 1H, H3'), 4.42-4.40 (m - 5 lines, H3"), 4.32-4.28 (m, 1H, H4'), 4.08-4.04 (m, 1H, H4"), 4.03-4.00 (m, 2H, H5"a and H5"b), 3.76-3.73 (m, 2H, H5'a and H5'b), 3.44 (s, 1H, acetylenic H), 2.50-2.20 (m overlapping, 4H, H2'a, H2'b, H2"a and H2"b).

The product was assayed for phosphorous according to procedure of B.N. Ames, Methods Enzymol. 1966, 8, 115-118.

Example 4

Method

(a) Animal Dosing and 5-FU Urinary Sampling

5-FU and 5-EU bases and nucleosides were dissolved in deionized HPLC grade water immediately prior to dosing. All animals were dosed once, p.o. via gastric gavage at the indicated mg/kg dose for each compound (See Results Section and Figures). 5-EU and 5-EU nucleosides were dosed to animals 0.5 hr prior to dosing with 5-FU and 5-FU nucleosides. Dosing was routinely

- 27 -

performed between 11am and 1pm. An approximate volume of 300 μ L was used for each dose. Rats were placed in individual Nalge metabolism cages, which separate urine and faeces, and continued on a 12 hr light/dark schedule post-dosing. Mice were treated post-dose in a similar manner with the exception that 5 mice were housed per cage.

Urine from each cage was collected into 0 - 24 hr and 24 - 48 hr post-dose fractions. Cages were rinsed with deionized water prior to collection of each fraction. The combined volume of urine and rinse was brought to a standard volume with deionized water.

(b) Quantitation of 5-FU by Reversed-Phase HPLC Analysis.

5 ml of a thoroughly mixed urine sample was filtered through a Millex GS 0.22 μ m filter (Millipore, Bedford, MA) and frozen prior to HPLC analysis. 1 ml of the thawed primary filtered solution was diluted with 2 ml of water and particulates were removed from this diluted stock solution using a Centrifree micro partition system (Amicon Division, W. R. Grace and Co., Beverly MA). 100 μ L of this solution was quantitated for 5-FU by HPLC on a Waters Associates System (Waters Associates, Milford, MA) equipped with a Waters 600E system controller, a Waters 600 solvent delivery system, a Waters model 712 WISP automated sample injector, a Waters 484 tuneable absorbance detector, and a Waters 991 photodiode array detector. For quantitation of 5-FU in rat urine, HPLC was performed on a 250 mm x 4.6 mm YMC AQ-303 S-5 120A ODS analytical column (YMC, Morris Plains, NJ) or on a Microsorb C18 analytical column with identical dimensions (Rainin Instrument Co., Woburn, MA). For each analysis the columns were first eluted isocratic at 0.5 ml/min with aqueous 50mM ammonium acetate buffer, pH 4.8, and 0.5% acetonitrile (Buffer A) for 25 min, followed by a linear gradient over 15 min to a 50% mixture with aqueous 50mM ammonium acetate buffer, pH 4.8, and 60% acetonitrile (Buffer B). Over the next 5 min a linear gradient was run to 100% buffer B, followed by isocratic elution with buffer B for 20 min. A linear gradient was then eluted across the column over a 10 min period to 100% buffer A and held at a flow rate of 0.5 ml for 2 min. The column was then re-equilibrated in buffer A at a flow rate of 0.75 ml/min for 15 min and then

- 28 -

returned to the original flow rate (0.5 ml/min) for 2 min prior to injection of the next sample. The effluent was monitored at 266 nm and 5-FU had a retention time of ca. 16.5 min. Data was collected on the Waters 991 diode array detector and a Digital Specialties microcomputer equipped with an in-house HPLC data analysis program (CHROM). The UV peak areas were integrated on these systems and compared to those of a standard curve prepared from known aqueous concentrations of 5-FU isocratically eluted across the Microsorb column with Buffer A. The molar extinction coefficient used for 5-FU was $7070 \text{ M}^{-1}\text{cm}^{-1}$ at a λ_{max} of 266 nm.

Plots of UV peak area vs 5-FU concentration were linear between 5 μM and 100 μM . The total urinary recovery of 5-FU was determined by multiplication of the nmoles of 5-FU determined from the standard curve by the dilution factor of 3 (above) and by the standard volume of urine collected.

5-FU recovery in mouse urine was determined as above with three exceptions: a Phenomenex 5 μm Extrasil C-18 column (250mm x 4.6 mm i.d.; Phenomenex, Torrance, CA) was used; an identical gradient with buffer A as aqueous 50 mM formic acid, pH 3.5, with 0.5% acetonitrile and buffer B as aqueous 50 mM formic acid, pH 3.5, with 60% acetonitrile was used; the standard curve was prepared by diluting known concentrations of 5-FU into undosed mouse urine and eluting with the latter described gradient and buffers, instead of isocratic conditions.

Results

(a) Release and Quantification of 5-FU From Nucleosides.

Rats were predosed with 5-EU (2 mg/kg, p.o.) 0.5 hr prior to dosing with the 5-FU nucleosides as shown in Table 1. The 5-FU nucleosides were dosed at either 10 or 25 mg/kg. The arabinoside and 2',3'-dideoxyriboside required the higher dosing in order to detect and quantify urinary recovery of 5-FU. For all nucleoside examples, 5-FU was only detected in the 0 - 24 hr urine and not in the 24 - 48 hr urine sampling. The riboside and 2'-deoxyriboside are optimum *in vivo* releasers of 5-FU with a urinary recovery of 5-FU > 65%.

Table 1

Urinary Recovery of 5-FU In Rats For Various 5-FU Containing Nucleosides

	Dose (mg/kg)	Predose	% 5-FU Recovery 0-24 hr Urine (Average of Two Experiments)
5-FU	5	-	<5
5-FU	5	2	52
5-FU	25	0.03	43-63
5-FU Nucleosides			
Riboside	10	2	78
2'-deoxyriboside	10	2	67
Tegafur	10	2	38
5'-chloro-2',5'- dideoxyriboside	50	0.03	21
Arabinoside	25	2	6
2',3'-dideoxy riboside		2	<5

(b) Protection of 5-FU From DPD Catabolism by Release of 5-EU From Nucleosides.

As seen in Fig 1, rats were orally predosed with 0.03 mg/kg 5-EU and with 0.03, 0.3 and 3.0 mg/kg of various 5-EU nucleosides, prior to dosing with 25 mg/kg 5-FU. 5-FU was observed and quantitated in only the 0 - 24 hr urine samples. Approximately 65% of the 5-FU dose was recovered unchanged in the urine for the 5-EU treated rats. Predosing rats with either the riboside or the 2'-deoxyriboside of 5-EU (≥ 0.03 mg/kg) allowed for high urinary recovery (>40%) of 5-FU. A direct dose response for urinary recovery of 5-FU was observed when the arabinoside or the 2'-deoxy-5'-monophosphate of 5-EU was used to deliver the DPD inactivator. Urinary recovery of 5-FU was <5% at all

- 30 -

predoses of 2', 3'-dideoxy-5-ethynyluridine.

(c) Urinary Recovery of 5-FU in Rats Dosed with the Succinate Linked Nucleoside

Rats were dosed 25 mg/kg 5-FU equivalent with the succinate linked nucleosides. Greater than 35% of the 5-FU in the original dose was recovered unchanged in the 0 - 24 hr urine. 5-FU was not detected in the 24 - 48 hr urine.

(d) Urinary Recovery of 5-FU in Mice Dosed with the Succinate linked Nucleosides.

For these studies mice were dosed with either 5 mg/kg 5-FU or given an equivalent dose of 5-FU via the succinate linked nucleoside. The urinary recovery of 5-FU was measured in the presence and absence of 5-EU (Table 2). Without predosing mice with 5-EU before delivering the base 5-FU, 5-FU could not be detected in the urine. When mice were predosed with 5-EU followed by dosing with 5-FU, the urinary recovery of 5-FU was 50 %. Mice dosed with the succinate linked nucleoside gave 25 - 30% urinary recovery of 5-FU available from the linked compound. There was no improvement of 5-FU urinary recovery from mice that were predosed with 5-EU followed by dosing with the succinate linked nucleoside.

Table 2

Dose 5mg/kg equivalent 5-FU	Predose	Recovery 5-FU (%)
5-FU	-	0
5-FU	2mg/kg	51
5-Ethynyl-5"-fluoro-3,3'"-O-succinylbis(1-(2-deoxy- β -D-erythropentofuranosyl)uracil	-	28
5-Ethynyl-5"-fluoro-3,3'"-O-succinylbis(1-(2-deoxy- β -D-erythropentofuranosyl)uracil	2mg/kg	23

Example 5

Antitumour Studies

Method

In vivo antitumor testing with murine Colon 38 was conducted in-house. C57BL/6 female mice were housed in sterile, polycarbonate, filter-capped Microisolator cages (Lab Products, Inc., Maywood, NJ) containing sterile hardwood bedding. Mice were fed sterile rodent chow and filter-sterilised water ad libitum. All manipulations of these mice were conducted in laminar-flow biosafety hoods. Colon 38 carcinoma was obtained from the Development Therapeutics Program Tumour Repository. Mice were implanted s.c. with 70 mg tumour fragments using a number 13 trocar. There were 10 mice in the control group that received tumour implants without drug treatment. There were 5 mice in each group treated with the dose and compounds as described below. Treatment began on day 16 post tumour implant and compounds were dosed once/day for 9 days. 5-FU was dosed at 15, 20, 25, 30, and 40 mg/kg.. The succinate linked nucleoside was dosed at 4.5, 8.9, 17.9, 26.8, 35.7 and 44.6 mg/kg. The molecular weight of the succinate linked nucleoside is 580.40 and the corresponding equivalents of 5-FU that were dosed with this compound were 1.0, 2.0, 4.0, 6.0, 9.0 and 10.0 mg/kg. A sample of 5-FU and the succinate linked nucleoside doses are indicated in Table 3. Tumour weights were calculated three times per week from measurements of tumour length and width. Antitumor activity was expressed as the days delay in tumour growth (T-C). T-C was calculated as the difference in the median of days for treated animals minus control animals for tumour mass to double twice post initial day of dosing (Day 16). Tumour-free survivors were not excluded from T-C calculations. Surviving mice were sacrificed after day 56.

Results

Antitumor Efficacy of 5-ethynyl-5"-fluoro-3,3'"'-O-succinylbis(1-(2-deoxy- β -D-erythropentofuranosyl)uracil)

The effect of the succinate linked nucleoside was studied in mice with s.c. implants of Colon 38 vs therapy with 5-FU alone. Antitumor activity was expressed as tumour growth delay (T-C; see Methods). The succinate linked nucleoside was toxic at doses > 17.9 mg/kg (4 mg/kg (4 mg/kg 5-FU and 5-EU equivalents). Table 3 shows a sub-optimum, effective and toxic dose for 5-FU and the succinate linked nucleoside.

Table 3

Compound	Dose (mg/kg 5-FU equivalent)	Antitumour Activity T-C (Days)	Tumour-free Survivors (Day 56)
5-FU	25	14.9	0/5
5-FU	30	23.6	0/5
5-FU	40	Toxic	0/5
*	1	12.9	0/5
*	2	>27.9	2/5

* \equiv 5-Ethynyl-5"-fluoro-3,3'"'-O-succinylbis(1-(2-deoxy- β -D-erythropentofuranosyl)uracil)

CLAIMS

1. A compound of the formula



wherein X represents a uracil reductase inactivator or prodrug thereof, Y represents 5-fluorouracil or a prodrug thereof, and L is a linking group covalently linked to both X and Y.

2. A compound according to claim 1 wherein X is a 5-substituted or 5,6-dihydro-5-substituted uracil derivative, the 5-substituent being bromo, iodo, cyano, halo-substituted C₁₋₄ alkyl, C₂₋₆ alkenyl, halo substituted C₂₋₆ alkenyl group, a C₂₋₆ alkynyl group, halo substituted C₂₋₆ alkynyl group.

3. A compound according to claim 1 or 2 wherein X is selected from

5-ethynyluracil
5-propynyluracil
5-cyanouracil
5-bromoethynyluracil
5-(1-chlorovinyl) uracil
5-iodouracil
5-hex-1-ynyluracil
5-vinyluracil
5-trifluoromethyluracil, and
5-bromouracil.

4. A compound according to any one of claims 1 to 3 wherein X is 5-ethynyluracil or 5-propynyluracil.

5. A compound according to any one of claims 1 to 4 which is:

5-ethynyl-5"-fluoro-3',3'''-O-succinylbis-(1-(2-deoxy-β-D-erythropento
furanosyl)uracil);

- 34 -

5-ethynyluridine-5-fluoro-1-(β -D-arabinofuranosyl)uracil succinic acid 5',2'-diester;

5-fluoro-5''-(1-propynyl)-2',2'''-O-succinylbis(1- β -D-arabinofuranosyluracil);
and

5'-chloro-2'-5'-dideoxy-5-fluorouridine 2'''-deoxy-5''-ethynyluridine (3'-5''') diphosphate.

6. A compound of formula (II) or (III)



wherein X, L and Y are as defined in claim 1 and n is at least 2.

7. A compound of formula (I), (II) or (III) as hereinbefore defined for use in medicine.
8. The use of a compound of formula (I), (II) or (III) as hereinbefore defined in the manufacture of a medicament for cancer chemotherapy.
9. A method for the treatment of cancer in a mammal comprising administering an effective amount of a compound of formula (I), (II) or (III) as hereinbefore defined to said mammal.
10. A method of reducing or inhibiting the tumour burden comprising administering to a mammal in need thereof an effective amount of a compound of formula (I), (II) or (III) as hereinbefore defined
11. A pharmaceutical composition comprising a compound of formula (I), (II) or (III) as hereinbefore defined together with at least one pharmaceutically acceptable carrier or excipient, and optionally one or more other therapeutic ingredients.

- 35 -

12. A pharmaceutical composition according to claim 11 for oral administration.
13. A method for the oral administration of 5-fluorouracil, comprising administering to a mammal in need thereof an effective amount of a compound of formula (I), (II) or (III) as hereinbefore defined.
14. A process for the preparation of a compound of formula (I), (II) or (III) as hereinbefore defined comprising reacting a uracil reductase inactivator or prodrug thereof, or a monoprotected derivative of a uracil reductase inactivator or prodrug thereof, with a compound L'-Y wherein Y is as hereinbefore defined or a protected derivative thereof and L' is a group capable of reacting with the uracil reductase inhibitor to form the linking group L, followed by deprotection where necessary.
15. An intermediate of formula L'-Y wherein L' and Y are as hereinbefore defined.

1/1

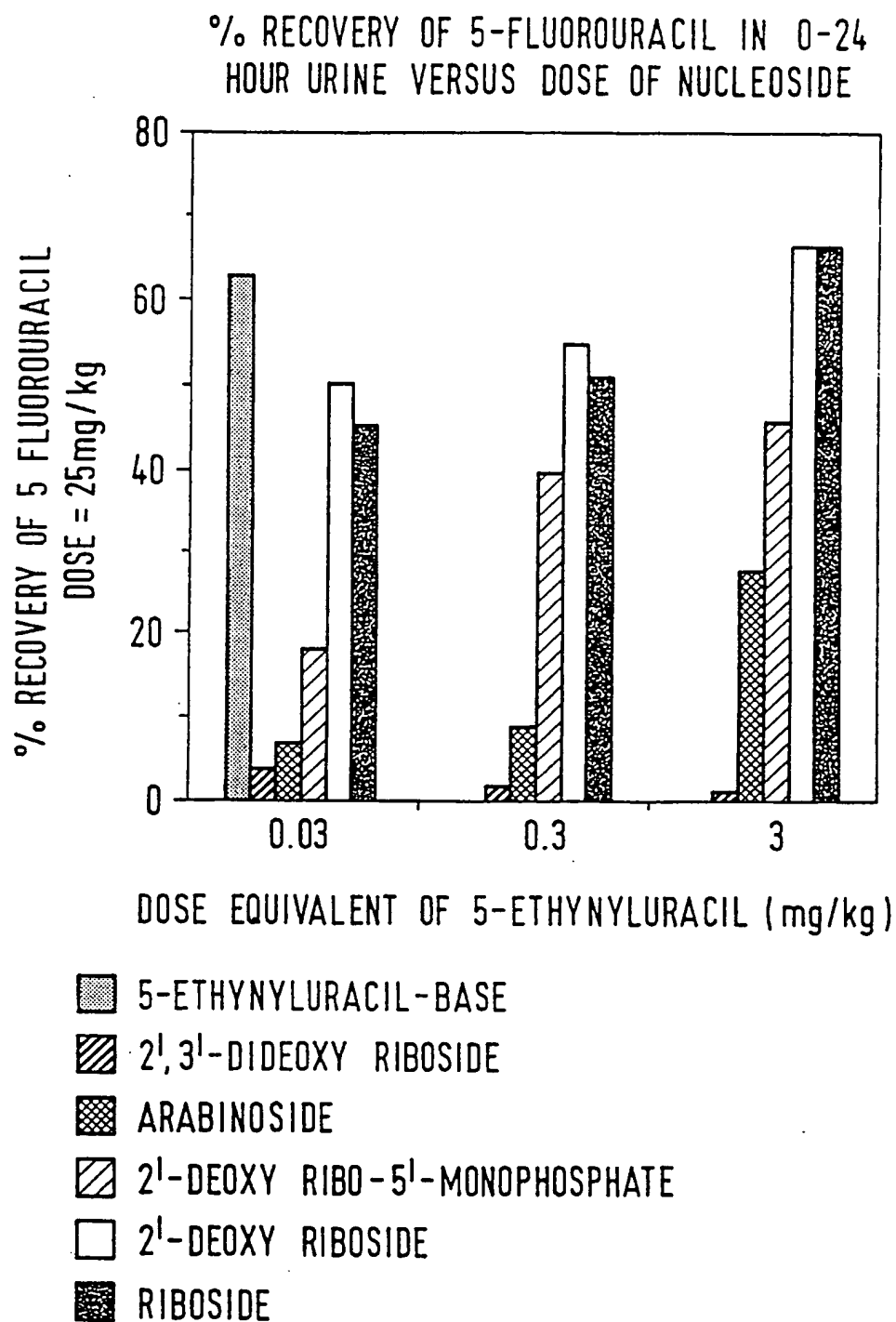


Fig. 1

SUBSTITUTE SHEET (RULE 26)

INTERNATIONAL SEARCH REPORT

Inter. Application No
PCT/GB 94/02428

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07H19/06 C07D239/54 A61K31/70 A61K31/505

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07H C07D A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, vol.146, no.3, 1987 pages 1270 - 1276 H.KORONIAK ET AL. 'The Structural Study of Internal (2+2) Photodimers of the Derivatives of 1,1'-Trimethylen-bis(5-fluoro) Uracil.' see the whole document ---	1-15
Y	WO,A,92 04901 (THE WELLCOME FOUNDATION LIMITED) 2 April 1992 cited in the application see the whole document ---	1-15
Y	WO,A,92 01452 (THE WELLCOME FOUNDATION LIMITED) 6 February 1992 ---	1-15
	--- -/--	

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

- * "A" document defining the general state of the art which is not considered to be of particular relevance
- * "E" earlier document published on or after the international filing date
- * "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- * "O" document referring to an oral disclosure, use, exhibition or other means
- * "P" document published prior to the international filing date but later than the priority date claimed

- * "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- * "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- * "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- * "&" document member of the same patent family

Date of the actual completion of the international search

7 February 1995

Date of mailing of the international search report

17 02 95

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl,
Fax (+ 31-70) 340-3016

Authorized officer

Scott, J

INTERNATIONAL SEARCH REPORT

Inter national Application No
PCT/GB 94/02428

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP,A,0 436 902 (OTSUKA PHARMACEUTICAL CO., LTD.) 17 July 1991 see page 2, line 10 - line 15 see abstract ---	1-15
Y	DE,A,16 20 631 (THE UPJOHN COMPANY) 12 February 1970 see page 3 see pages 5 and 6, structures (j),(k),(l) and (m) ---	1-15
Y	GB,A,2 066 812 (FUJI KAGAKU KOGYO K.K.) 15 July 1981 see abstract -----	1-15

INTERNATIONAL SEARCH REPORT

International application No.

PCT/GB94/02428

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 9, 10, 13
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 9, 10, 13 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☒ Claims Nos.: 1-15
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

See Appended sheet
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

LIMITED SEARCH ON BROAD MAIN CLAIM

1).The wording "Uracil reductase inactivator" (cl.1, p.33, 1.3) seeks to define the invention by a result to be achieved. Claim 1 is directed to a compound, but the present wording tries to define part of the claimed compound by structural features (i.e. 5-fluorouracil) and another part by a result to be achieved. It does not provide any structural information with regard to X (the uracil reductase inactivator) at all. Furthermore, it is not clear whether X alone or X-L or indeed X-L-Y act as the uracil reductase inactivator.

2).The wording "Or prodrug thereof" (cl.1, 1.3 and 4) is vague and potentially even unlimited. It attempts to extend the scope of the present invention in some vague and undefined manner. In accordance with the exemplified embodiments in the description, and a reasonable set of variations on them, the word prodrug thereof has been restricted to uracil and uridine derivatives.

3.i).The retrieved, prior art can also be used to demonstrate the lack of ready clarity with regard to this terminology.

3.ii).D1 = EP - A - 0 436 902
D2 = GB - A - 2 066 812

3.iii).D1 discloses (p.2,1.10-15) 5-fluorouracil and 5-fluorouridine derivatives which are substituted at the 3'- or 5'- position by groups optionally having specific substituents and related compounds via a phenyl lower alkyloxy linker. More specifically, pyridine derivatives are attached to the 5-FU derivatives via the linker. These compounds have been shown to exhibit improved antitumour effects and this may be due to the fact that the pyridyl-substituents attached to the linker-5FU moiety are inactivating uracil reductase.

3.iv).Similarly, D2 details 5-fluorouridine attached to nitrogen containing groups via an acyl linker. These compounds have improved activity/toxicity ratios - which may be due to the nitrogen containing groups inactivating uracil reductase.

3.v).Both these documents could be viewed as destroying both the novelty and inventivity of claim

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

1, if the wording "Uracil reductase inactivator" and "Or prodrug thereof" is used.

4). In accordance with the last paragraph of Chapter III, point 3.6 of the PCT Search Guidelines, the subject-matter of claim 1 has not been exhaustively searched. The only subject-matter of claim 1 with respect to X that has been exhaustively searched is that which is further defined in claim 2, directed to preferred compounds of claim 1 (and taking into account the objection to the wording "Or prodrug thereof" - point 2 above).

5). In this perspective, starting from the plethora of possible individual compounds and without making any assumptions which would have no basis in the original disclosure, it is not possible to unambiguously determine a (or a plurality of) generalised set(s) of distinct features which could be considered as special technical features of a solution or of a plurality of alternative solutions to an accordingly formulated problem underlying the invention as a whole.

6). Consequently, claim 1 does not comply with Article 6 and Rule 6.3(a) PCT, which require that an invention should be clearly defined in terms of features supported by the description. These features should be identifiable by means of an appropriate technical statement of the problem and its solution.

7). The application does not comply with Rule 5.1(a)(ii) and (iii) in that the description does not indicate the common feature(s) of the compounds embraced by the broad definition of claim 1 in a manner sufficient for understanding the invention and carrying out the search.

8). For the same reasons, claim 6 has only been searched on the basis of the preferred embodiments listed in claim 2.

9). The search Claims 14 and 15 have only been searched within the scope of the examples actually outlined in the present application.

The wording "Uracil reductase inhibitor" and "Or prodrug thereof" are objected to for the reasons outlined in points 1 and 2 above.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

The definition of L' seeks to define as a physiological group which yields a product when reacted with another group which is not clearly defined. Furthermore, this definition concerns a "Uracil reductase inhibitor" and not an "Inactivator" as mentioned elsewhere in the claims. This begs the question, does reacting a uracil reductase inhibitor with L'-Y cause it to become a uracil reductase inactivator, and if so, what part do L' and Y play in the uracil reductase inactivation ?

Thus it is not possible to search these claims except when read in the light of the exemplification presented in the application.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter. Application No

PCT/GB 94/02428

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9204901	02-04-92	AU-B- 654505	10-11-94
		AU-A- 8540591	15-04-92
		CA-A- 2092435	27-03-92
		CZ-A- 9300506	16-02-94
		EP-A- 0550580	14-07-93
		JP-T- 6504263	19-05-94

WO-A-9201452	06-02-92	EP-A- 0539442	05-05-93
		JP-T- 6502619	24-03-94

EP-A-0436902	17-07-91	JP-B- 1057118	04-12-89
		JP-C- 1570217	10-07-90
		JP-A- 61106593	24-05-86
		DE-A- 3583726	12-09-91
		DE-A- 3585869	21-05-92
		EP-A, B 0180188	07-05-86
		EP-A, B 0180897	14-05-86
		EP-A- 0323441	05-07-89
		US-A- 4983609	08-01-91
		US-A- 5155113	13-10-92
		US-A- 4864021	05-09-89
		JP-A- 63301880	08-12-88
		JP-A- 62155215	10-07-87
		JP-B- 1053878	15-11-89
		JP-C- 1570237	10-07-90
		JP-A- 62149696	03-07-87

DE-A-1620631	12-02-70	CH-A- 490393	15-05-70
		FR-A- 1481649	
		GB-A- 1149261	
		GE A- 1149262	
		NL-A- 6606669	02-12-66
		US-A- 3317512	02-05-67

GB-A-2066812	15-07-81	JP-C- 1394624	11-08-87
		JP-A- 56077298	25-06-81
		JP-B- 62001399	13-01-87
		JP-A- 56092299	25-07-81
		CH-A- 646441	30-11-84
		DE-A, C 3044740	24-09-81

information on patent family members

PCT/GB 94/02428

Form PCT/ISA/210 (patent family annex) (July 1992)